

SOME OBSERVATIONS ON THE EGGS OF *FUCUS* AND UPON THEIR MUTUAL INFLUENCE IN THE DETER- MINATION OF THE DEVELOPMENTAL AXIS

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Several species of the seaweed *Fucus* are obtainable in abundance on the rocky shores of Nahant peninsula, outside the entrance to Boston Harbor. These seaweeds have a long breeding season, shedding eggs and sperm in the winter months when other types of eggs are difficult to obtain. In 1929-30 eggs of *Fucus vesiculosus* were collected from October until June. Throughout this time some eggs were always obtainable from plants with relatively large fruiting tips, or receptacles, and in February, March, April, and May they were obtainable in great abundance.

Fucus vesiculosus is dioecious. The sexes can be separated by sight with fair reliability if a cut receptacle is examined. The conceptacles of the male plants are orange due to the carotinoids contained in the antherozoid or sperm cells. The conceptacles of the female plants are green or brownish-green, largely because of the plastids in the eggs. Identification becomes certain if a thin section of the receptacle is examined microscopically.

The purpose of this paper is to present a number of observations and experiments, some of which have been incidental to measurements on respiration in the *Fucus* eggs which will be presented elsewhere. The results recorded here have particularly to do with the nature of the eggs and with certain factors involved in determining the first division plane. The first division of the *Fucus* egg ordinarily gives rise to two cells of different shape. One, which includes the rhizoidal protuberance, is the parent cell for the formation of the rhizoid, the other gives rise by divisions to the thallus. At the first division, therefore, the polarity or developmental axis of the spore has been determined and is first indicated. A number of environmental factors have been found capable of determining the cleavage plane and the polarity of the *Fucus* spore. Orientation of the cleavage by a directed beam of light has been demonstrated in a number of plants (*c.g.*, Pierce, 1906). Farmer and Williams (1898) have shown that if fertilized *Fucus* eggs are illuminated

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from one side, the rhyzoids usually originate on the side of the egg remote from the light. Miss Hurd (1920) found that in *F. inflatus* (a monoecious species, collected in San Francisco Bay) when directed beams of different light frequencies are used, red light has no effect but the short blue orients the cleavage and the direction of growth of the rhyzoid.

At Friday Harbor Lund (1923) passed an electric current through sea water containing spores of the monoecious *Fucus inflatus*. Cross streams of sea water were designed to carry off electrolytic products formed at the electrodes. A potential drop of 25 millivolts across the diameter of an egg oriented the division plane and the developmental axis. The rhyzoid cell came to lie toward the + pole.

There are no visible marks or identifications of polarity in the unfertilized *Fucus* egg. The nucleus lies in the middle of the cell. When spores which are not too greatly crowded develop in the dark, the directions of divisions lie entirely at random. Miss Hurd (1920) observed in her work with colored light that if the eggs lie close together, within 2 or 3 egg diameters, they tend to send out the rhyzoid toward each other, or toward the center of a nearby mass of eggs. This phenomenon, which she calls a "group effect," was especially pronounced in the dark, but was strong enough to overcome the orienting effect of the directed light if the eggs were close together.

This directive effect of one egg on another at a distance presents some points of interest. It might possibly be due to a differential of oxygen tension, or of CO₂, or to the accumulation of some other metabolite. If the cells are either giving off or consuming some substances in radial fashion, in the case of neighbors the additive effect on the intervening space between them would cause that part of the sea water environment to be most altered. No jelly or solid substance of the eggs traverses this space. Winkler (1900) attempted to establish a gradient of oxygen tension across the spores of *Cystosira barbata*, which are also oriented by light, to see if this might be the determining factor. The results were negative. In view of recent work purporting to show an effect at a distance upon dividing cells due to "mitogenetic" radiation, the possibility of some such effect in the "group effect" in *Fucus* presents itself. Mitogenetic rays have been supposed to affect primarily the division rate of neighboring cells, rather than the polarity or plane of division. It is possible that this qualitative distinction is not justified, however, as the plane of division may be determined by asymmetric or differential rates of the processes leading to cell division. The experiments which are to be described do not discover the nature of this influence of cells at a distance. It is at present possible to answer the

following two questions: 1. In order that cells shall exert this influence upon a neighbor, must there be nuclear activity or cell division in the directing cells? 2. Is the directive effect in *Fucus* specific? The answers to these questions still leave open the question of a possible rôle of mitogenetic rays, since this type of radiation has been as well demonstrated coming from non-dividing tissues, even recently macerated tissue, as from dividing cells. A brief review of some of this work is given by Hollaender and Schoeffel, 1931. Further experiments to continue these preliminary observations are planned.

Before proceeding to a consideration of these experiments, however, a description of the *Fucus* eggs, and the results of some other observations, will be presented.

The Gametes of Fucus

The *Fucus* plant is diploid. As in animals the haploid generation is confined to the gametes. Yamanouchi (1909) has estimated the chromosome number in *F. vesiculosus* to be 64-32. After the plants had been brought into the laboratory the sex of each plant was determined by microscopical examination of a section of a receptacle. The receptacles were then cut off and placed in covered glass dishes in an ice box at about 3° C. Usually within 24 hours or less the gametes within their capsules had been extruded from the receptacles to lie in mounds on the outside of the receptacles. Removal of the plants from sea water induces shedding. In nature the shedding is stimulated when the plants are stranded at low tide. Too much drying, however, is highly detrimental, and it is best to keep the receptacles in covered dishes as well as to keep them cool.

The eggs lie 8 in a capsule when shed. The capsule wall consists of two thin membranes, probably with a gelatinous substance between, 10 microns wide. When $KI + I_2$ is added, and then H_2SO_4 , the capsule membranes and the space between them do not turn blue (although the eggs within do). This indicates that the capsule is not made of cellulose. When HNO_3 and NH_3 are added these membranes, and especially the space between them, turn deep yellow-brown, indicating that they are proteinaceous. The membranes coagulate and partly dissolve when the acid is added, and some coagulation of the substance between the membranes is observed. More slowly the eggs also turn yellow, although the color of the plastids tends to some extent to obscure the color.

When the egg capsules are washed off from the fruiting tips into normal sea water, after a time, depending on temperature, first the outer and later the inner membrane of the capsule breaks and the eggs are aborted into the sea water, often being somewhat squeezed together in

passing out. If the eggs are kept cold, they apparently undergo no deterioration for many days. High percentages of fertilization and of normal spores have been obtained from eggs which had remained unfertilized, either in light or dark, for more than a week.

Many diflagellate sperm or antherozoids occur in small capsules (64 antherozoids per capsule, according to Yamanouchi, 1909). These capsules are exceedingly delicate and appear to dissolve completely in the sea water. The sperm are immotile when liberated, but they begin to move in a few seconds, and are soon swimming actively. My own experience has been that their life is short, or rather that they do not fertilize eggs well after a few hours, especially if they have been in dilute suspension. They may be kept a number of days, however, if they remain dry on the fruiting tips in a moist chamber. They are immotile in this condition. Sperm suspensions of even moderate concentration are brilliant orange.

The unfertilized eggs of *Fucus vesiculosus* vary considerably in size. Single eggs varied between 52 and 70 microns in diameter, averaging between 60 and 65. Larger eggs are found which will be discussed later. The eggs are readily stratified by centrifuging, and develop normally after stratification. Eggs centrifuged immediately after fertilization tend to become ameboid but eventually round up. Centrifuging for 20 minutes at 2600 r.p.m. (20 cm. radius, 18° C.) throws almost all of the formed bodies to one end. A gray cap occupies the end position. Its thickness is about one ninth the diameter of the egg. The nucleus lies at its inner edge, just between it and the plastids, which are densely crowded into a zone bordering the gray cap. A few plastids remain behind adhering to the peripheral regions of the egg. The stratified materials are all less dense than the cytoplasm at large, as the gray cap is seen to float uppermost when the eggs settle in a tube and are observed from the side as they fall. The eggs are comparatively dense. They settle more rapidly than the eggs of such animals as *Arbacia*, *Cumingia*, *Chaetopterus*, etc., in spite of their smaller size. The volume of materials which are moved by the centrifuge is much less in proportion than in such animal eggs as the sea urchin's. Since the eggs are photosynthetic they are perhaps able to dispense with as large a store of food materials.

The unfertilized eggs may readily be cut with the microneedle, although unless they are pinched gently with care, they burst and disperse their substance into the sea water. They appear to be only poorly prepared to gelate a new surface on an exposed cut, being deficient in materials for what Heilbrunn has called the "surface precipitation reaction."

Fertilization

When a heavy sperm suspension is added to unfertilized eggs, the eggs are rapidly rotated by the sperm. After a few minutes they stop rotating even though the sperm are still fully active. That this whirling of the eggs has no significance in bringing about fertilization is evident (1) because dilute sperm suspensions which fertilize the eggs cause no rotation, and (2) because concentrated sperm of another member of the *Fucaceæ*, *Ascophyllum nodosum*, which do not fertilize the eggs of *Fucus vesiculosus* rotate the *Fucus* eggs more rapidly than the eggs of their own species (which are larger).

Fertilized eggs were placed in a thermostat at 18° C., and the time-lapse to the first division was noted. The time span over which first divisions occur in a population is a wide one, covering approximately 13 to 18 hours. Fifty per cent of the eggs were observed to have divided after 14 hours in one case, and after 15 hours in another. At cold temperatures the time is greatly extended.

The *Fucus* egg has been described as secreting a cellulose wall immediately after fertilization. Eggs were fertilized and then cut with the microneedle at increasing intervals after fertilization, to determine the physical nature of this secretion and the sequence of physical change. It has been stated that the unfertilized eggs may readily be cut in half, although they burst very easily. When they burst no membrane of any sort is visible around the egg or remaining behind. Five minutes after fertilization the eggs may be cut with the greatest ease. There is no longer any tendency to burst. When the fragments are separated with the needle a sticky gelatinous material, which has been secreted, is seen to bridge across between the fragments. At 10 minutes conditions are about the same. Fifteen minutes after fertilization a slight increase in the rigidity of this gelatinous secretion is noticeable. After 30 minutes it is slightly more firm. At 45 minutes it has become a definite semi-solid wall which holds its shape but which is still easily cut. After an hour the secretion has become so firm as to be cut only with great difficulty, and usually only after bursting the egg within. After an hour and a half the wall is tough and rigid. The eggs dodge the needle and cannot be cut.

It is probably this sticky secretion which causes the eggs to adhere to the substrate and stop rotating after fertilization. The eggs adhere to the substrate with increasing tenacity until by 2½ hours they are fairly well attached, or if they are kept in suspension by gentle shaking, after 2½ hours they are clumped together in adhering masses. After the secreted wall has become firm the eggs are well protected and may at any

time be dislodged without damage from the substrate to which they adhere.

The secreted wall of the fertilized egg was subjected to colorimetric cellulose and protein tests. The walls of 24-hour spores gave no protein reaction, although of course the egg itself and cytolytic extrusions from it turned deep yellow. The cell walls turned brilliant blue when subjected to $KI + I_2 + H_2SO_4$, a test for cellulose. No rotation of polarized light was observed with a polarizing microscope, but this may well have been because the cellulose is too thin to cause a detectable amount of rotation.

The unfertilized eggs show a faint blue color in the cellulose test, perhaps due to the material in the cortex which is to be secreted. Ten minutes after fertilization the color reaction of the secreted jelly is equally faint. At 25 and 55 minutes there is a slight increase in the blue reaction. At about an hour and a half the first decidedly blue color results. Even this, however, is not as brilliant a blue as in the 24-hour eggs. It seems probable that a relative of cellulose is secreted as the sticky jelly, and that gradually the crystal molecular arrangement is assumed so that by an hour and a half the tough cellulose wall has been formed, which gives the blue color reaction. As the rhizoid grows out on one side this membrane must be softened and added to; at any rate it precedes and sheathes the early rhizoid. Nevertheless it gives a brilliant blue color reaction at the tip as if it does not reconvert back into the faintly coloring jelly.

If eggs which have been fertilized for an hour and a half are placed in sea water saturated with dextrose they shrink and collapse. Unfertilized eggs do not collapse. Eggs which have been fertilized for 25 minutes do not collapse, while those which have been fertilized for 45 minutes collapse to some extent. Eggs which have been fertilized for 3 hours or longer collapse and leave behind the transparent cellulose wall, which is clearly revealed in this way. Farmer and Williams note that placing the eggs in tap water so that they burst and flow out also reveals the cell wall. The difference in behavior in dextrose sea water is often a convenient and fairly reliable way of testing for fertilization, as the jelly and the cellulose wall are not themselves readily seen in the normal fertilized egg. After two and a half hours in dextrose-saturated sea water the eggs have mostly rounded out and recovered their shape. Shrinking with sugar in the later stages causes the individual cells of the spore to stand out clearly. Even the early spores have a remarkable ability to withstand dehydration and to develop normally after being returned to normal sea water. Thus embryos one hour, an hour and a half, and two hours after fertilization were placed in dextrose-saturated

sea water for two hours. They were then returned to normal sea water, and developed in typical fashion.

The Origin and Fate of Giant Eggs

The size of single eggs of *Fucus vesiculosus* eggs varies considerably (about 52–70 microns). In addition, a number of giant eggs, often much larger, are frequently found in a sample. The proportion of these giants depends greatly on the treatment to which the eggs have been subjected before their emergence from the capsules. In some cases more than half will be giants, most of which are much larger than the eggs seen within the capsule. This difference in size led Behrens, 1886 (cited by Farmer and Williams, 1896), to propose that the large eggs represent a stage in fertilization. Farmer and Williams (1896, 1898) point out that by no means are all fertilized eggs larger than the unfertilized eggs in the capsules, and they further noted that two or three nuclei are sometimes visible in these large eggs, which they therefore regarded as abnormal eggs. Examples of what appear to be giant eggs appear in photographs by Hurd (1920) and Lund (1923). It might be supposed that these large multinucleate eggs result from the failure of certain of the parent germ cells to divide. While this may possibly be the case sometimes, careful direct observations have shown another and simple origin of frequent occurrence.

When the eggs come out of the capsule they are often pressed together. At this time they often fuse to form giants. These giants have been observed to form as the result of the fusion of 2, 3, 4, 5, 6, 7, and 8 single eggs. The giant egg, of course, contains the corresponding number of nuclei, although possibly these fuse later. Once eggs have fused, they have never been seen to separate later. The effect of temperature at the time of emergence is very marked. Samples of the same set of capsules were divided into lots, some of which were placed at 3° C. and some at between 25° C. and 28° C., during the period of break-down of the capsules and release of the eggs. At the lower temperature the break-down of the capsules takes a much longer time, but after the eggs were out this striking difference was found: In the eggs at 3° C. only 3 giants were found in about 6000 eggs. In the eggs which had emerged at 25–28° C. between 50 per cent and 60 per cent of all the eggs were giants, mostly of about 4 fused eggs. The high temperature apparently alters what might loosely be called the consistency of the eggs, so that they are much more prone to fuse. One of the benefits of keeping the eggs cold while they are emerging from the capsules is to avoid these giants in experiments in which they are to be

avoided. The plants should be kept cold from the time of collecting, as otherwise some fusions will take place within the capsules.

The development of these giants involves some peculiarities and variations. No doubt they occur to some extent in nature when the eggs are shed on warm days. Many hundreds of individual eggs were isolated and fertilized, and single mononucleate eggs were never observed to undergo abnormal cell division nor to give rise to more than one rhizoid (although this in some cases branched at a later time). The giants also usually divided in normal fashion, with one giant rhizoid, but not infrequently two and sometimes three independent rhizoids developed simultaneously from various parts of the egg. The number of rhizoids bore no special relation to the number of cells which had fused to form the giant egg, except that there were never more rhizoids than component cells. Thus, isolated giants which had been seen to originate from the fusion of six eggs (for example) formed in some cases one, in others two, and occasionally three rhizoids. Most commonly when two rhizoids formed they grew out side by side, sometimes having a common basal part. Not infrequently, however, when reared in the dark and away from neighbors, two rhizoids grew out 180° or less apart. A few examples are sketched in Fig. 1.

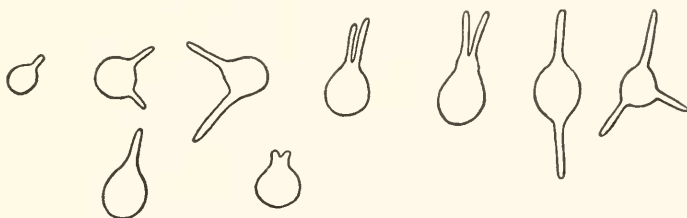


FIG. 1. Spores from fused eggs (*F. vesiculosus*). The small left-hand spore is a single egg.

Sometimes one of the supernumerary rhizoids, especially if it is small, may disintegrate and sluff off, while the remainder of the spore then develops normally.

The causes which determine whether twinning, or what degree of twinning, shall take place in these giant spores are not known. Two possibilities suggest themselves. It may depend upon how many of the constituent egg cells receive sperm independently, or it might depend upon the degree of fusion of the constituent cells, as determining whether the egg nuclei may come together to form a single polyploid nucleus, or whether internal partitions (former cell surfaces) persist, holding the nuclei apart so that they become independent division-centers. At any rate, the relation between the twinning and the fused origin of the

giant eggs indicates that the phenomenon is of the same general sort as the duplication in parts which results from experimentally fused eggs or blastulae of sea urchins and starfishes (cf. Morgan's text, 1928).

Cross-Fertilization

In the spring gametes were obtained from the monoecious *Fucus evanesceus*.² The eggs and sperm are shed simultaneously in a heavy mass of viscous jelly which covers the entire fruiting tip. These eggs are larger than those of *F. vesiculosus*, averaging about 85–90 microns in diameter. They also differ in color, being reddish-brown instead of brownish-green. The immature eggs of *F. evanesceus* within the conceptacles are quite green, indicating that the reddish-brown pigments develop in the eggs as they approach maturity. Later in the spring (in April and May) gametes from the dioecious *Ascophyllum nodosum* were also obtained in abundance. The eggs of this form are green. They vary in diameter about between 60 and 85 microns, and occur four in a capsule instead of eight.

Experiments were designed to see if cross-fertilizations will take place among these three species. Fruiting tips were washed in fresh water to kill any antherozoids which might be upon them, and were dried with filter paper and then dipped in filtered sea water to restore the normal salt environment. They were then placed in individual moist chambers to shed.

Eggs and sperm from a given fruiting tip of *F. evanesceus* often exhibited 99 per cent fertilization. It is apparently not necessary for the sperm of different individuals to be exchanged for the highest percentages of fertilization, as in some hermaphroditic animals such as the ascidian *Ciona* (Morgan, 1924).

Cross-fertilizations were attempted as follows: *F. vesiculosus* sperm \times *Ascophyllum* eggs, *F. vesiculosus* eggs \times *Ascophyllum* sperm, *F. vesiculosus* eggs \times *F. evanesceus* sperm. In all cases samples of the eggs and sperm used were also tested against the corresponding gametes of the same species as a control to be sure that they were in good condition. Samples of *F. vesiculosus* and *Ascophyllum* eggs were also run as unfertilized controls to check against contamination. Since *F. evanesceus* eggs could not be obtained separately from sperm no attempts were made to fertilize them with foreign sperm. The *F. evanesceus* sperm could be obtained free from eggs by drawing off sea water above *evanesceus* eggs which had settled in a dish. *F. vesiculosus* sperm were added to *Ascophyllum* eggs in four experiments. In the first experi-

² I am indebted to Professor W. R. Taylor for the identification of this species from a pressed specimen.

ment about ten per cent of the eggs divided, but also about ten per cent of the unfertilized controls developed. Either contamination or parthenogenesis occurred. The experiment was repeated three more times with large dishes of eggs with no further development whatsoever. The controls were good in these experiments. *Ascophyllum* sperm were added to *F. vesiculosus* eggs in three experiments. In two cases no divisions took place. In the third case three eggs in about 3,000 divided. The controls were all good. *F. vesiculosus* eggs were inseminated with *F. evanesens* sperm in ten experiments. The controls were all good. The percentages of development in the ten experiments were as follows: 1 per cent, 1 per cent, 0.1 per cent, 0, 0, 0, 0, 0, 0, 0.

The conclusion must be that less than 1 per cent cross-fertilization takes place among these forms in normal sea water.

The "Group Effect"

In the course of some other experiments large numbers of dishes of *F. vesiculosus* eggs had been reared in the dark and many examples were seen of what appeared to be division of eggs so that the rhizoids pointed toward near neighboring spores. Since it would not be difficult to be deceived, with neighboring cells in all directions, some sixty dishes were prepared in which only two eggs were placed between one and three egg-diameters apart. These were reared in the dark and were later examined microscopically with an apparatus which made it unnecessary to jar or touch the dishes. The results were then tabulated, dividing the circle around each egg into quadrants, one of which included the angle 45° to either side of the line joining the two eggs. Counts were made of the eggs whose rhizoids protruded in each quadrant. The count showed an entirely random distribution. There was no correlation between the division plane and the position of the neighboring cell. This result was very surprising as some very convincing signs of correlation had been seen in dishes containing many cells. Accordingly, dishes were prepared in which a large compact mass of eggs lay in the center of the dish, and around about the periphery of the mass isolated eggs were placed at intervals well apart and about two egg diameters out from the peripheral cells of the mass. In this configuration there is no ambiguity as to the direction of the neighboring cell mass as in a random scattering over the bottom of a dish. The eggs were reared in the dark. The results in these cases were as clear-cut as in those with only two eggs in a dish, but they were quite the opposite. Some actual counts of the directions in which the rhizoids protruded from these outlying cells are as follows: Within the 180° of arc toward the central mass 257 eggs, in the 180° away from the mass 2 eggs; in 57 eggs, out

1 egg; in 340, out 7; in 40, out 3 etc. The peripheral cells of the central mass itself also divided with rhizoids inward in comparable proportions. It appears then that some general condition resulting from large numbers of eggs in the dish is necessary in order that several neighbors shall have



FIG. 2. Outline sketch of a group of *F. evanescent* eggs reared in the dark, showing the "group effect." Some of the inner cells have divided equally without producing rhizoids.

this mutual effect. The causes of this were not worked out. For the present purpose it was sufficient to find a circumstance in which the "group effect" invariably takes place. This condition is met when eggs are placed around a central mass of hundreds or thousands of eggs in a Syracuse dish, regardless of the shape of the mass. In very small dishes a smaller mass may suffice.

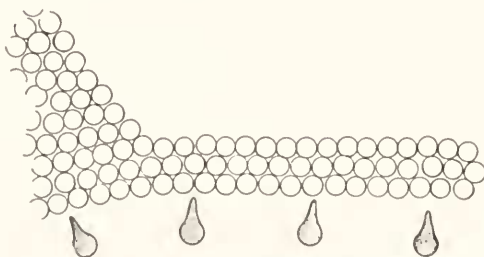


FIG. 3. Sketch of part of an experiment, showing *F. evanescent* spores (stippled) directed by unfertilized *F. vesiculosus* eggs. These spores were reared in the dark, and *F. evanescent* eggs are on one side only of the *F. vesiculosus* mass.

The eggs of *Ascomyllum* also exhibit the "group effect" and the eggs of *F. evanescent* show it very markedly, much more markedly than the eggs of *F. vesiculosus*. That is to say, even small isolated groups of these eggs alone in a dish develop rhizoids pointing toward the nearest neighbor, or in the resultant direction if there are a number of neighbors. Rings of four or five eggs have the rhizoids all pointing inward. In large masses not only the peripheral eggs have rhizoids pointing inward, but often five or six outer layers of cells also all point inward. In masses of eggs of this species, especially larger masses, the

innermost cells not infrequently divide into two equal and similar cells instead of producing one rhyzoid cell. Farmer and Williams observed occasional equal divisions in *F. vesiculosus* eggs, especially when reared in the dark. Some actual counts of the direction of the rhyzoid in peripheral cells of masses of *F. evanescent* eggs (again dividing the circle into two divisions of 180°) are as follows: in toward center of mass 260, out 8; in 38, out 0; in 113, out 3; in 210, out 2 etc. An example of a small isolated cluster of these eggs is shown in Fig. 2.

To answer the two questions raised in the first part of this paper, masses of closely-packed *F. vesiculosus* eggs were arranged in Syracuse dishes and in smaller dishes, and with a mouth pipette eggs of *F. evanescent* were placed at intervals around the periphery of each mass.

TABLE I

The results of seven experiments showing the directive effect of unfertilized *F. vesiculosus* eggs on the developing spores of *F. evanescent*.

Experiment No.	In	Out	Tangent	Equal Division
1	36	1	0	1
2	41	0	1	2
3	12	0	1	0
4	14	1	0	0
5	8	0	1	1
6	10	0	0	0
7	12	0	0	0
Totals	133	2	3	4

The *F. evanescent* eggs were placed at distances varying between one half and two egg diameters out from the periphery of the *F. vesiculosus* mass. Around the circumference the distance between consecutive *F. evanescent* eggs ranged from five to a hundred or more egg diameters, to rule out any effect which they might have on each other. (Other tests showed that no directive influence extended beyond four or five egg diameters.) To further control against any mutual effect of the *F. evanescent* eggs, in some cases the *F. evanescent* eggs were placed only on one side of the mass of *vesiculosus* eggs so that no other *evanescent* eggs whatsoever, at any distance, would be in the direction of the central *vesiculosus* mass. (Fig. 3.)

When newly shed and fertilized *F. evanescent* eggs were added in place, supernumerary *F. evanescent* sperm also came into the dish, but these did not fertilize the *F. vesiculosus* eggs of the central mass. The dishes were then placed in a dark room under black felt and were later examined without jarring the dishes. Since the fertilized spores adhere

to the bottom of the dish they are not easily dislodged, but the unfertilized *vesiculosus* eggs are easily moved.

The results of seven experiments are given in Table I. In the column headed "in" are represented the eggs whose rhizoids pointed inside an imaginary line tangent to the edge of the central mass. "Out" represents those which pointed outside the tangent line. "Tangent" represents the eggs whose rhizoids paralleled the tangent line. "Equal" represents eggs which divided equally, without producing a rhizoid. All four cases of equally dividing eggs developed cell plates parallel to the tangent line, as if to protrude rhizoids directly inward (or out).

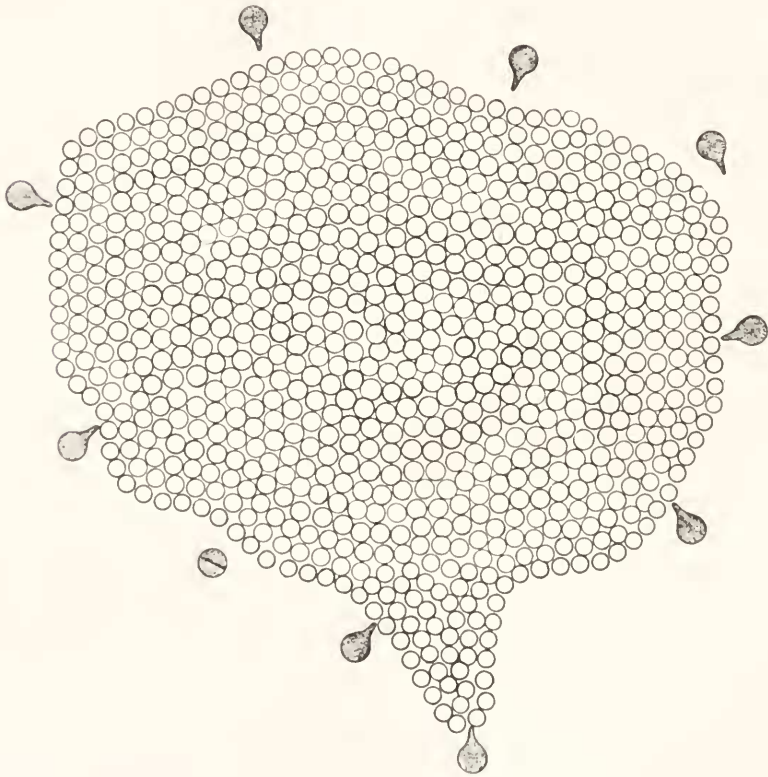


FIG. 4. The central mass consists of unfertilized resting *F. vesiculosus* eggs. At intervals around the periphery are directed *F. evanescent* spores, which are stippled. Their greater size has been slightly exaggerated. Reared in the dark.

The great preponderance of spores which divided with rhizoids within the tangent line did so with the rhizoid pointing almost exactly toward the nearest cell of the central mass. In experiments one, five, and six, the *F. evanescent* eggs were placed on only one side of the central mass.

In experiments two, three, four, and seven, they were placed all around the mass. The direction of the central mass from the *evenescens* eggs therefore covered all points of the compass and controlled against environmental asymmetries such as, for example, stray light (which, however, was not present). In experiments one and two the central mass was about one centimeter across; in experiments three, four, five, six, and seven, it was about two or three millimeters across, although the shape of the mass was not always strictly circular. A sketch of one of these experiments is given in Fig. 4. The peripheral *evenescens* cells are placed more closely together in this experiment than in the others.

Discussion of the Directive Agency in the "Group Effect"

The results show conclusively that eggs of *F. evenescens*, which tend to divide so that the rhizoid grows in the resultant direction of neighboring cells, are equally well directed by unfertilized resting eggs of another species as by dividing eggs of the same species. The directive effect therefore cannot be ascribed to any agency dependent on nuclear or cell division in the directing cells. The effect is also non-specific, although the two species tested are of the same genus.

I am much indebted to Professor W. J. Crozier, in whose Laboratory these experiments were performed, for advice and criticism, and to Professor W. H. Weston and Dr. A. E. Navez for advice and help in locating the habitat of the *Fucus* and in the proper handling of the plants in order to obtain gametes. Professor W. R. Taylor has been so kind as to identify *F. evenescens* for me from a pressed specimen.

SUMMARY

1. The results of a number of observations and experiments are presented which relate to the nature of the *Fucus* egg and to some of the changes which take place in it at fertilization.
2. Giant eggs which in some cases develop supernumerary rhizoids are found to originate in the fusion of single eggs within the capsule and especially at the time of emerging from the capsule.
3. The extent to which eggs fuse is found to be greatly reduced at low temperature and increased at high temperature.
4. Cross-fertilization between *F. vesiculosus* and *Ascophyllum nodosum* was found not to take place. The sperm of *F. evenescens* do not fertilize the eggs of *F. vesiculosus* to any appreciable extent. Individual receptacles of the monocious *F. evenescens* are entirely self-fertile.

5. The developmental axis of the spores was found to be directed by the presence of nearby neighbors in *F. vesiculosus*, *F. evanescent*, and in *Ascophyllum*. The first division plane tends to lie so that the rhizoid protrudes in the resultant direction of near neighbors. No jelly or other solid egg substance traverses the space between affected eggs.

6. Unfertilized resting eggs of another species were found to direct the division planes of eggs of *F. evanescent* equally well as dividing eggs of the same species. Therefore the directive effect cannot be ascribed to any agency dependent on nuclear or cell division in the directing cells.

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